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In Vitro Vascular Cell Adhesion and Proliferation on Alkaline Degraded Poly-lactic/glycolic Acid Polymers

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ABSTRACT

The objective of the present in vitro study was to determine vascular endothelial and smooth muscle cell responses to poly(lactic-co-glycolic acid) (PLGA) films that were exposed apriori to various degrees of alkaline degradation. To model the alkaline environment of blood in arteries, PLGA films were separately soaked in select concentrations (from 0.1 – 10 N) of NaOH for various periods of time (from 10 minutes to 1 hour). Vascular endothelial and smooth muscle cells were then separately allowed to adhere and/or proliferate on the different PLGA degraded surfaces. Results provided the first evidence that smooth muscle adhesion and proliferation increased with larger amounts of alkaline PLGA degradation. In contrast, endothelial cell adhesion and proliferation decreased with increasing amounts of alkaline PLGA degradation. In this manner, the present in vitro study suggests a possible mechanism for insufficient endothelialization on PLGA vascular implants in vivo.

INTRODUCTION

Biodegradable polymers (such as poly(lactic acid), poly(glycolic acid), and poly(lactic-co-glycolic acid)) have become attractive candidates in vascular tissue engineering [1-4]. Such polymeric scaffolds can be easily shaped into grafts to serve as three-dimensional substrates capable of promoting vascular tissue ingrowth. Moreover, it is hoped that these biologically resorbable scaffolds will dissolve in situ and leave behind a regenerated neo-vascular conduit. Unfortunately, to date, polymer scaffolds containing poly(lactic acid) have not lived up to their potential [1, 2]. Formation of fibrovascular tissue in the intima of implanted grafts often leads to intimal hyperplasia which has resulted in occlusion of the regenerated vascular tissue [1, 2]. Since an endothelial cell lining often fails to develop on the luminal surface of polymers that contain poly(lactic acid), no regulatory mechanisms exist to minimize fibrovascular tissue ingrowth [1]. Clearly, the inability of poly(lactic acid) containing polymers to promote sufficient endothelialization presents serious limitations for this polymer as a successful vascular prosthetic material [1, 2]. The goal of this research was to determine, for the first time, in vitro vascular endothelial and smooth muscle cell responses (specifically, adhesion and proliferation) to poly(lactic-co-glycolic acid) at various stages of alkaline degradation, similar to the degradation which would be experienced in vivo, in order to better understand cellular events that lead to previously observed [2] insufficient endothelialization.

MATERIALS AND METHODS

Substrates

Poly(lactic-co-glycolic acid) (PLGA)

PLGA (50/50 weight % poly(lactic acid)/poly(glycolic acid), Polysciences, Inc.) samples were prepared by dissolving (at 50-60 °C) 0.5g of PLGA in 8ml chloroform. This solution was poured into glass petri dishes, allowed to sit overnight, and then transferred to a vacuum (15mm Hg) oven for 2 days at room temperature. The resulting film was cut into either 0.5 cm x 1 cm or 1 cm x 1 cm strips. The polymer strips were treated in three ways to simulate various stages of biodegradation: untreated (non-degraded); 0.1 N NaOH for 10 minutes (partially degraded); and 10 N NaOH for 1 hour (extensively degraded). Polymer strips were sterilized by exposure to UV light for 2 hours followed by soaking in ethanol for 24 hours.

Glass

Borosilicate glass coverslips (Fisher) were used as a reference substrate in experiments with cells. Coverslips were degreased in acetone and ethanol, etched in 10 N NaOH for 1 hr, and sterilized in an autoclave before use [5].

Cells

Rat aortic smooth muscle cells and rat aortic endothelial cells were purchased from VEC Technologies and used without further characterization. Smooth muscle cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% Fetal Bovine Serum (FBS, Hyclone) and 1% Penicillin/Streptomycin (P/S, Hyclone), while endothelial cells were cultured in MCDB-131 Complete Medium (VEC Technologies). All cells were maintained under standard cell culture conditions (that is, a sterile, humidified, 95% air, 5% CO_2, 37 °C environment). Population numbers (for both cell lines) used in experiments were between 6 and 10.

Experiments

Adhesion on Polymer Substrates

Endothelial and smooth muscle cells were seeded separately (3500 cells/cm^2 in either MCDB-131 Complete Medium or DMEM supplemented with 10% FBS and 1% P/S, respectively) onto various PLGA films and were allowed to adhere for 4 hours under standard cell culture conditions. Non-degraded (untreated) polymer substrates were used as controls.

Proliferation on Polymer Substrates

Endothelial and smooth muscle cells were seeded separately (3500 cells/cm^2 in either MCDB-131 Complete Medium or DMEM supplemented with 10% FBS and 1% P/S, respectively) onto various PLGA films and were allowed to proliferate for 1, 3, and 5 days under standard cell culture conditions. Non-degraded (untreated) polymer substrates were used as controls.

Staining and Counting

At the end of the prescribed time periods, all substrates were rinsed in phosphate buffered saline to remove non-adherent cells, fixed in 10% formalin, and stained with 0.1% Coomassie Brilliant Blue. The number of cells in each of five random fields per substrate were counted using a light microscope, averaged, and recorded as cell density (cells/cm^2). Experiments were run in triplicate and repeated at least three separate times.

RESULTS

Adhesion on PLGA

Compared to non-degraded (untreated) PLGA, vascular endothelial cell adhesion decreased significantly ($p < 0.05$) on the extensively degraded PLGA formulations after 4 hours (Figure 1). Specifically, endothelial cell density was two times less on the extensively degraded, compared to non-degraded, PLGA. In contrast, smooth muscle cell adhesion was significantly ($p < 0.05$) enhanced on extensively degraded PLGA compared to non-degraded PLGA after 4 hours (Figure 1). Cell density ranged from 1,036 cells/cm^2 for the non-degraded PLGA to 1,433 cells/cm^2 for extensively degraded PLGA.

Proliferation on PLGA

Compared to non-degraded (untreated) PLGA, results provided evidence that endothelial cell proliferation decreased ($p < 0.05$) on extensively degraded PLGA compared to non-degraded PLGA at every time point tested in the present study (Figure 2). For example, after 5 days of culture, the number of endothelial cells decreased by approximately one half on extensively degraded compared to non-degraded PLGA. In contrast, smooth muscle cell proliferation increased ($p < 0.01$) on extensively degraded PLGA formulations after 3 and 5 days of culture (Figure 3). Specifically, the number of smooth muscle cells was two and four times greater on extensively degraded compared to non-degraded PLGA after 3 and 5 days, respectively.

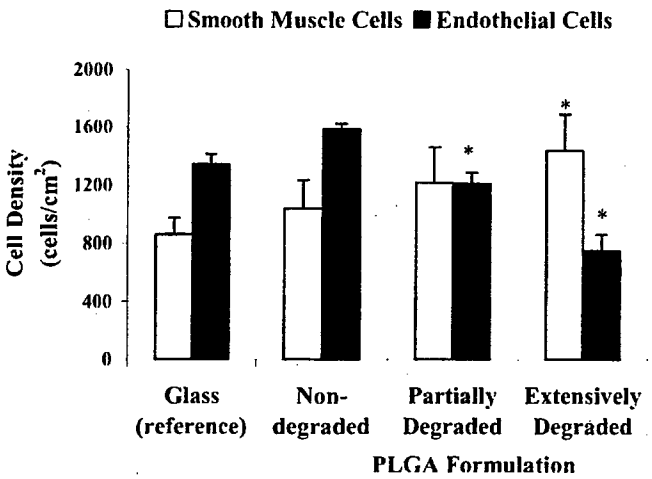

Figure 1. Enhanced Smooth Muscle Cell and Decreased Endothelial Cell Adhesion on Extensively Degraded PLGA. Data are mean +/- SEM; n = 3; * $p < 0.05$ (compared to respective adhesion on non-degraded, or untreated, PLGA).

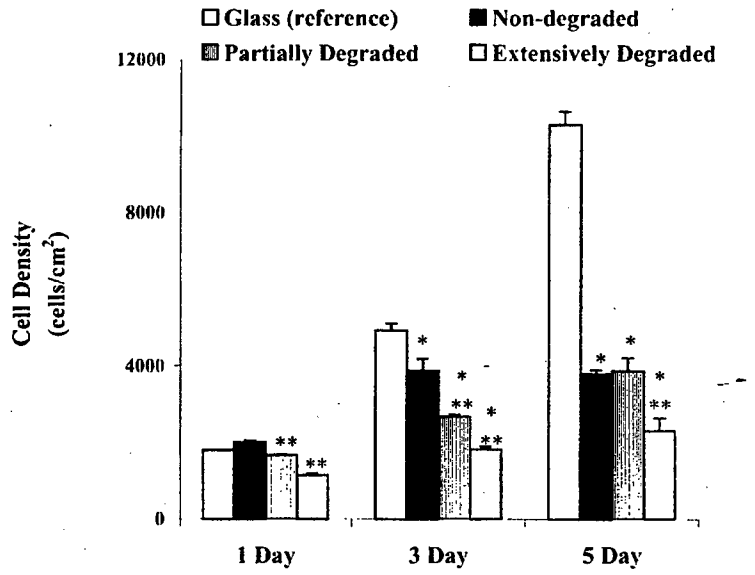

Figure 2. Decreased Endothelial Cell Proliferation on Extensively Degraded PLGA. Data are mean +/- SEM; n = 3; * $p < 0.01$ (compared to proliferation on respective PLGA after 1 day); ** $p < 0.05$ (compared to proliferation on non-degraded, or untreated, PLGA at respective day).

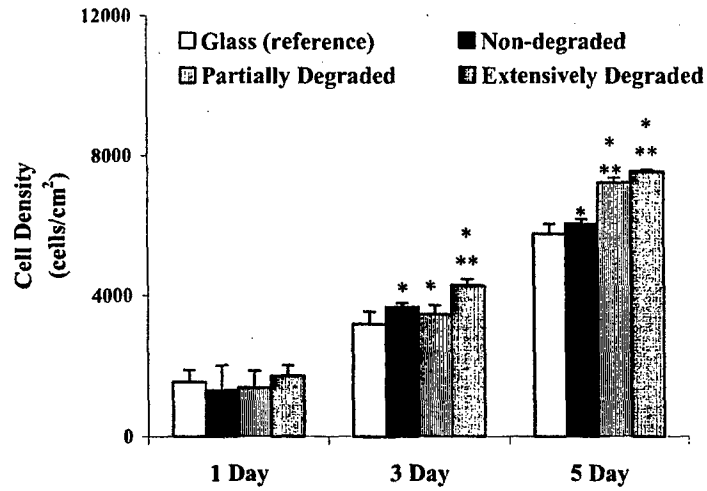

Figure 3. Enhanced Smooth Muscle Cell Proliferation on Extensively Degraded PLGA. Data are mean +/- SEM; n = 3; * $p < 0.01$ (compared to proliferation on respective PLGA after 1 day); ** $p < 0.05$ (compared to proliferation on non-degraded, or untreated, PLGA at respective day).

DISCUSSION AND CONCLUSIONS

The present study sought to model alkaline degradation of PLGA as would be experienced by this polymer when exposed to blood in arteries [6]. This was accomplished by soaking PLGA in select concentrations of NaOH for specific periods of time, thereby developing a partially degraded and extensively degraded PLGA film. Gao et al. [4] previously described the hydrolysis procedure by which poly(glycolic) acid (PGA) meshes degrade in an alkaline environment. Briefly, hydroxide anions from NaOH hydrolyze the ester bond on the surface of the PGA mesh, thereby exposing carboxylic acid and hydroxyl groups by breaking the polymer chain. This process happens at various locations in the polymer leading to multiple-chain hydrolysis depending upon access of hydroxide ions to the polymer chains. Such hydrolysis may result in degradation of the polymer into oligomeric or monomeric forms whereby NaOH is able to further dissolve portions of the polymer fibers.

Since PLGA is also a poly (ester), we expected to observe similar interactions between the hydroxide ions of NaOH and the ester bonds of this polymer during the alkaline degradation events used in the present study. Results of endothelial cell experiments demonstrated, for the first time, that these cells adhere and proliferate better on non-degraded, compared to degraded, PLGA. In contrast, smooth muscle cells adhere and proliferate to a greater extent on degraded rather than non-degraded PLGA. Increased spreading of vascular smooth muscle cells have been

previously observed on PGA treated with NaOH [4]. However, this is the first study to report increased vascular smooth muscle cell adhesion and proliferation, as well as decreased functions of endothelial cells, on PLGA polymers treated with increasing concentrations of and exposure to NaOH. This information suggests that as PLGA degrades under alkaline blood conditions in the artery, smooth muscle cell functions will be enhanced while endothelial cell functions will be inhibited. Although it is not clear at the time whether altered vascular cell function is due to topographical or chemical changes in PLGA as alkaline degradation occurs, it is clear that successful endothelialization of degrading PLGA will require use of different polymer formulations.

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